DRUG EXTRAVASATION AND MICROKINETICS USING LASER SCANNING (CONFOCAL) MICROSCOPY

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The authors have developed novel methods for the visualisation of drug diffusion from capillaries into surrounding tissues in living tissue, and for quantifying and analysing these microkinetic events. The methods are being used to study:

i) Mechanisms and kinetics of macromolecule transport across capillary walls.
ii) Drug concentration profiles in the vicinity of capillaries and local capillary permeability.
iii) Rapid fluctuations of transcapillary concentration gradients over the the first few cardiac cycles after the administration of an intravenous bolus of drug.

Confocal microscopy (CFM) has been used to image fluorescent molecules arriving at a capillary within the inguinal fat pad of anaesthetised Wistar rats (pentobarbitone 60 mg/kg. I/P). A bolus of 200 µl. of 1.25% w/v fluorescein in 0.9%w/v NaCl in Water for Injections was administered via the jugular vein. The capillary and surrounding area were repeatedly scanned at a rate of one frame per second, using a 768x512x8 framestore (Synoptics,U.K.). This enabled extraction of concentration profiles within the capillary and in the extravascular space. Diffusion of the marker into adipocytes was minimal, and hence fluorescence in the extravascular compartment represented primarily extracellular material.

Arrival of the fluorescein at the capillary typically occurred four seconds after injection - the bolus could be observed to travel across the field of view during its first pass. By extracting a profile of fluorescence intensity along a line as indicated below, the monitoring of extravasation and extravascular diffusion of fluorescent compounds was possible.

Fig.1 Fluorescence intensity along line A-B in Fig.2

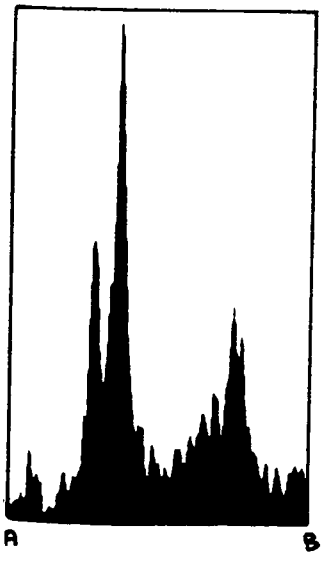


Fig.2 Image of capillary 4s after IV bolus

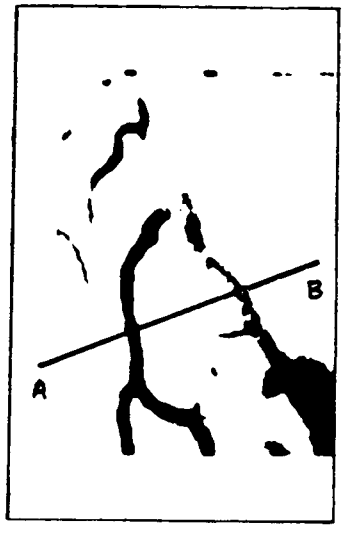
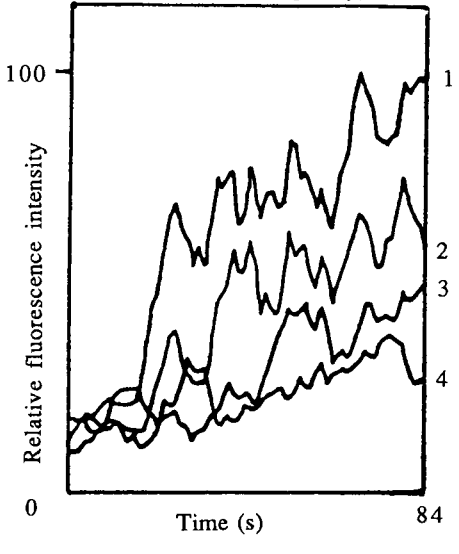


Fig. 3 Relative fluorescence intensity at four points in the tissue bed. Taken from an 84s time sequence at 1s intervals. (point 1 is nearest to capillary)



We have demonstrated a method which could be used to obtain pharmacokinetic data from within living tissue. Problems of poor diffusion across capillary walls, within tumour masses, and the effects of lymphatic drainage parallel the development of macromolecular drugs and delivery systems. The ability of CFM to measure drug concentrations in vivo and with high frequency and spatial resolution opens up the new field of microkinetics, which may prove to be of great significance in the development of novel drug delivery systems and in determining the physiological and therapeutic characteristics of macromolecular compounds.